Electronic Instruments for the Life Sciences

made to measure

OPERATING INSTRUCTIONS AND SYSTEM DESCRIPTION OF THE

VA-10M

VOLTAMMETRIC AND AMPEROMETRIC AMPLIFIER FOR EPMS SYSTEMS

with 8-pole BESSEL Filter

VERSION 4.1
npi 2014

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1. Safety Regulations

<u>VERY IMPORTANT</u>: Instruments and components supplied by npi electronic are NOT intended for clinical use or medical purposes (e.g. for diagnosis or treatment of humans), or for any other life-supporting system. npi electronic disclaims any warranties for such purpose. Equipment supplied by npi electronic must be operated only by selected, trained and adequately instructed personnel. For details please consult the GENERAL TERMS OF DELIVERY AND CONDITIONS OF BUSINESS of npi electronic, D-71732 Tamm, Germany.

1) GENERAL: This system is designed for use in scientific laboratories and must be operated by trained staff only. General safety regulations for operating electrical devices are to be followed.

2) AC MAINS CONNECTION: While working with the npi systems, always adhere to the appropriate safety measures for handling electronic devices. Before using any device please read manuals and instructions carefully.
   The device is to be operated only at 115/230 Volt 60/50 Hz AC. Please check for appropriate line voltage before connecting any system to mains.
   Always use a three-wire line cord and a mains power-plug with a protection contact connected to ground (protective earth).
   Before opening the cabinet unplug the instrument.
   Unplug the instrument when replacing the fuse or changing line voltage. Replace fuse only with an appropriate specified type.

3) STATIC ELECTRICITY: Electronic equipment is sensitive to static discharges. Some devices such as sensor inputs are equipped with very sensitive FET amplifiers, which can be damaged by electrostatic charge and must therefore be handled with care. Electrostatic discharge can be avoided by touching a grounded metal surface when changing or adjusting sensors. **Always turn power off when adding or removing modules, connecting or disconnecting sensors, headstages or other components from the instrument or 19” cabinet.**

4) TEMPERATURE DRIFT / WARM-UP TIME: All analog electronic systems are sensitive to temperature changes. Therefore, all electronic instruments containing analog circuits should be used only in a warmed-up condition (i.e. after internal temperature has reached steady-state values). In most cases a warm-up period of 20-30 minutes is sufficient.

5) HANDLING: Please protect the device from moisture, heat, radiation and corrosive chemicals.

2. EPMS-07 Modular Plug-In System

2.1. General System Description / Operation

The npi EPMS-07 is a modular system for processing of bioelectrical signals in electrophysiology. The system is housed in a 19" rackmount cabinet (3U) has room for up to 7 plug-in units. The plug-in units are connected to power by a bus at the rear panel.

The plug-in units must be kept in position by four screws (M 2,5 x 10). The screws are important not only for mechanical stability but also for proper electrical connection to the system housing. Free area must be protected with covers.

2.2. EPMS-07 Housing

The following items are shipped with the EPMS-07 housing:

- ✓ EPMS-07 cabinet with built-in power supply
- ✓ Mains cord
- ✓ Fuse 2 A / 1 A, slow
- ✓ Front covers

In order to avoid induction of electromagnetic noise the power supply unit, the power switch and the fuse are located at the rear of the housing.

2.3. EPMS-E-07 Housing

The following items are shipped with the EPMS-E-07 housing:

- ✓ EPMS-E-07 cabinet
- ✓ External Power supply PWR-03D
- ✓ Power cord (PWR-03D to EPMS-E-07)
- ✓ Mains chord
- ✓ Fuse 1.6 A / 0.8 A, slow
- ✓ Front covers

The EPMS-E-07 housing is designed for low-noise operation, especially for extracellular and multi channel amplifiers with plugged in filters. It operates with an external power supply to minimize distortions of the signals caused by the power supply.

2.4. PWR-03D

The external power supply PWR-03D is capable of driving up to 3 EPMS-E housings. Each housing is connected by a 6-pole cable from the one of the three connectors on the front panel of the PWR-03D to the rear panel of the respective EPMS-E housing. (see Figure 1, Figure 3). A POWER LED indicates that the PWR-03D is powered on (see Figure 1). Power switch, voltage selector and fuse are located at the rear panel (see Figure 2).

Note: The chassis of the PWR-03D is connected to protective earth, and it provides protective earth to the EPMS-E housing if connected.

Figure 1: PWR-03D front panel view

Figure 2: PWR-03D rear panel view

Note: This power supply is intended to be used with npi EPMS-E systems only.

2.5. System Grounding

EPMS-07

The 19" cabinet is grounded by the power cable through the ground pin of the mains connector (= protective earth). In order to avoid ground loops the internal ground is isolated from the protective earth. The internal ground is used on the BNC connectors or GROUND plugs of the modules that are inserted into the EPMS-07 housing. The internal ground and mains ground (= protective earth) can be connected by a wire using the ground plugs on the rear panel of the instrument. It is not possible to predict whether measurements will be less or more noisy with the internal ground and mains ground connected. We recommend that you try both arrangements to determine the best configuration.

EPMS-E-07

The 19" cabinet is connected to the PROTECTIVE EARTH connector at the rear panel. The chassis is linked to protective earth only if the PWR-03D is connected. It can be connected also to the SYSTEM GROUND (SIGNAL GROUND) on the rear panel of the instrument (see Figure 3).

Important:: Always adhere to the appropriate safety measures.

Figure 3: Rear panel connectors of the EPMS-E-07

2.6. Technical Data

19” rackmount cabinet, for up to 7 plug-in units
Dimensions: 3U high (1U=1 3/4” = 44.45 mm), 254 mm deep

EPMS-07

Power supply: 115/230 V AC, 60/50 Hz, fuse 2 A / 1 A slow, 45-60 W

EPMS-E-07

External power supply (for EPMS-E): 115/230 V AC, 60/50 Hz, fuse 1.6/0.8 A, slow

Dimensions of External power supply: (W x D x H) 225 mm x 210 mm x 85 mm

3. Introduction

Recently, electrochemical methods using carbon-fiber microelectrodes have been applied to measure the release of oxidizable transmitter from ***single cells***, and, even more impressively, from ***single exocytotic vesicles***. Transmitters that are oxidizable and which, therefore, can be measured with this approach, include serotonin, dopamine, adrenaline, and noradrenaline. In addition, some peptides or proteins such as insulin may be oxidizable owing to the presence of oxidizable amino acids such as cysteine or tyrosine.

Cells that have been studied successfully with this technique include adrenal chromaffin cells, sympathetic neurons, mast cells, pancreatic beta cells, carotid glomus cells and melanotrophs, but the list is growing. In addition, in brain slices simultaneous intracellular and voltammetric studies have been made to correlate intracellular electric signals with transmitter release.

Two useful electrochemical approaches are **amperometry** and **cyclic voltammetry**. In **amperometry**, a DC potential is applied to a carbon-fiber microelectrode. The applied potential appears at the interface between the carbon and the mammalian ringer solution. If the potential is much greater than the redox potential for a given transmitter, then molecules of transmitter diffusing to the carbon surface are oxidized rapidly yielding a current that can be measured. The sensitivity of the **amperometric** approach, in particular, has provided an unprecedented look at the time course of transmitter release revealing distinct phases of release. On the other hand, the amperometric approach provides little information about the substance being oxidized or reduced.

Cyclic voltammetry provides a limited amount of information about the substance being studied, at some expense to the time resolution. In this approach a cyclically repeating voltage waveform, typically consisting of voltage ramps, is applied to the carbon-fiber electrode and the resulting current is plotted as a function of the applied voltage (after subtraction of a "background" record obtained in the absence of the redox species). Since different substances have different potentials for oxidation and for reduction one can distinguish transmitters from each other.

For more detailed informations about the principles of electrochemical measurements at single cells and the fabrication of carbon-fiber microelectrodes refer to several recent reviews.

4. VA-10M Components

The following items are shipped with the VA-10 system:

- ✓ VA-10M amplifier
- ✓ Headstage
- ✓ GND connector for headstage (1 mm)
- ✓ User manual

Optional accessories:

- Carbon-fiber electrode holder
- Carbon-fiber electrodes (CFE), ∅ 5µm
- Headstage with differential input
- Four VA-10 amplifier system:
  Modified EPMS housing, 4 VA-10 amplifier modules, and a four-in-one headstage

5. VA-10M System

5.1. System Description

The VA-10M is a sensitive (picoampere range) current amplifier that is intended for voltammetric measurements with carbon-fiber microelectrodes in biological systems, where the total currents do not exceed 20 nA. It can be used for either DC amperometry using the built-in voltage source or it can be operated with user-supplied external voltage waveforms (e.g. for cyclic voltammetry).
The VA-10M is ideally suited for measurements with carbon-fiber disk microelectrodes having diameters of 10 µM or less from single cells plated onto glass cover slips. However, it can also be used for measurements made on superficially located cells in tissue slices. The VA-10M is not recommended for use in *in vivo* recordings with carbon-fiber electrodes having long cylindrical measuring surfaces, because in this case currents approach the µA range and a third electrode is required to compensate for the IR drop as currents flow through the extracellular fluid.

5.2. Description of the Front Panel

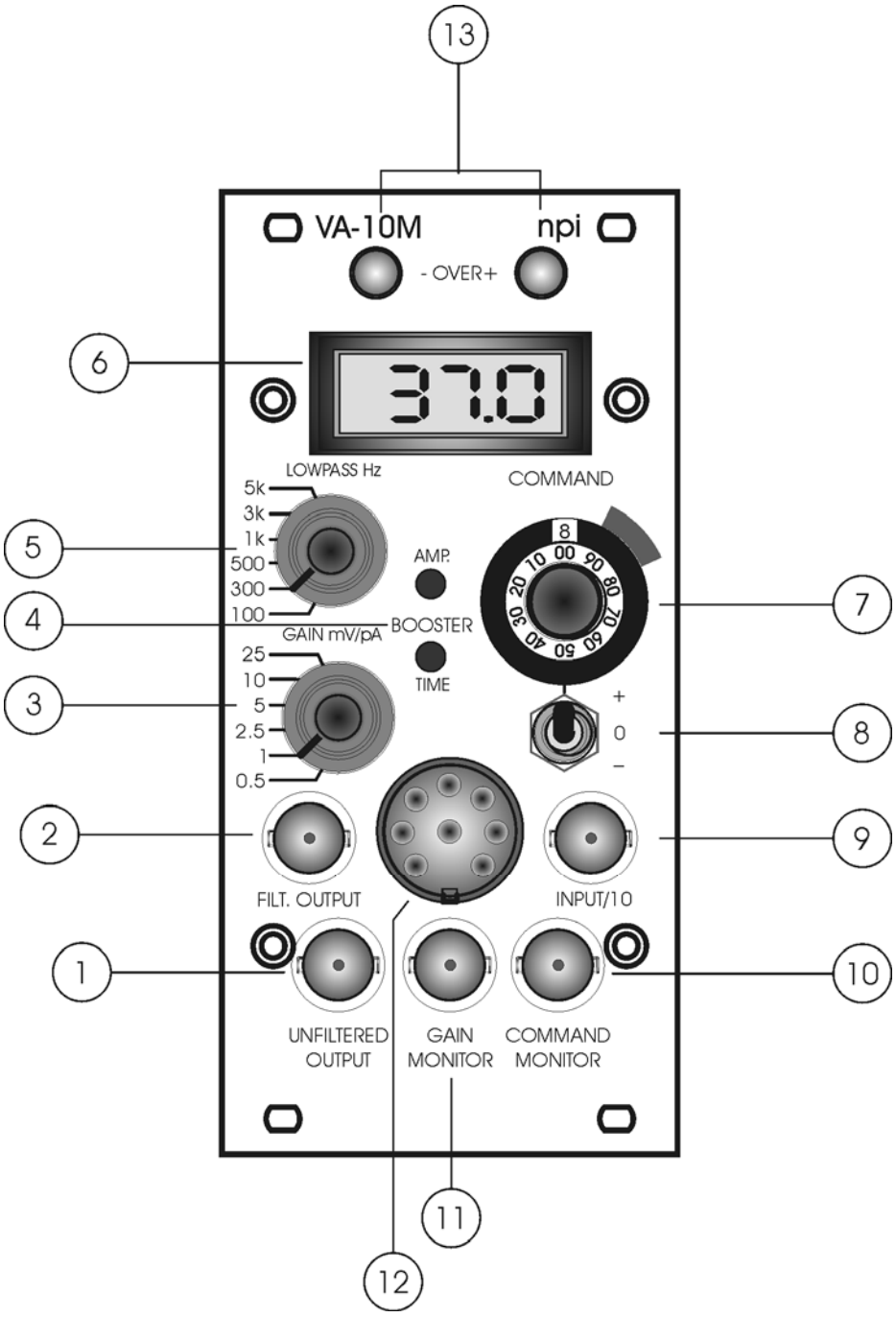

Figure 4: VA-10M front panel view

In the following description of the front panel elements each element has a number that is related to that in Figure 4. The number is followed by the name (in uppercase letters) written on the front panel and the type of the element (in lowercase letters). Then, a short description of the element is given.

(**1**) UNFILTERED OUTPUT connector

BNC connector providing an unfiltered voltage proportional to the current passed through the electrode. The signal is amplified by the GAIN factor selected by switch (**3**).

(**2**) FILT. OUTPUT connector

BNC connector providing a filtered voltage proportional to the current passed through the electrode. The signal is filtered by a low pass filter and amplified by the GAIN factor selected by switch (**3**). The corner frequency of the filter is selected by switch (**5**).

(**3**) GAIN mV/pA switch

6-position rotary switch to set the amplification factor (x0.5, x1, x2.5, x5, x10, x25 mV / pA) of the current proportional voltage signal at (**1**) and (**2**).

(**4**) BOOSTER

Trim pots for adjusting the FREQUENCY BOOSTER.

TIME: Trim pot for adjusting the TIME CONSTANT of the FREQUENCY BOOSTER.

AMP.: Trim pot for adjusting AMPLITUDE of the FREQUENCY BOOSTER.

Note: The BOOSTER is best adjusted by following the procedure described in chapter 7.2

(**5**) LOW PASS Hz switch

6-position rotary switch to set the corner frequency of the low pass 8-pole Bessel filter. The filtered OUTPUT can be obtained at connector (**2**).

(**6**) COMMAND voltage display

LCD that indicates the COMMAND voltage applied to the electrode (range: ±1000 mV, display: XXXX mV). The COMMAND voltage is set by potentiometer (**7**).

COMMAND unit

The COMMAND units consists of (**7**) COMMAND potentiometer and (**8**) +/0/- switch

(**7**) COMMAND potentiometer

10-turn potentiometer to set the command voltage in DC amperometric experiments using the internal voltage source (range: ±1000 mV).

(**8**) +/0/- switch

Switch to set the polarity of the COMMAND voltage. In position 0 the COMMAND voltage generated by the VA-10M is disabled.

(**9**) INPUT /10 connector

BNC connector to connect an external waveform for fast cyclic voltammetry. The INPUT voltage is divided by 10 internally and applied to the electrode.

Important: The voltage at the electrode is always the sum of the voltage at INPUT /10 connector and the setting at the COMMAND voltage potentiometer.

(**10**) COMMAND MONITOR connector

BNC connector providing the COMMAND voltage at the electrode multiplied by a factor of 10, i.e. the voltage is the sum of the setting at (**7**) COMMAND potentiometer times 10 and the voltage at (**9**) INPUT /10 connector.

(**11**) GAIN MONITOR connector

BNC connector that monitors the setting of (**3**) GAIN switch. Range: +1 to +6 V, resolution 1 V / STEP (i.e. 3V indicate a GAIN of 2).

(**12**) HEADSTAGE connector

8-pin connector for the HEADSTAGE cable.

Important: Always turn power off when connecting or disconnecting the headstage.

(**13**) OVER LEDs

LEDs indicating if the amplifier 10% below it's positive or negative limit (±10 V). The linear range of the amplifier is ±12 V.

6. Headstage

The VA-10M comes with the standard headstage (range: ±1000 mV) for connecting carbon-fiber electrodes via an electrode holder (optional).
A 3-electrode headstage with differential input (see also **Optional accessories** in chapter 4) is also available. For details contact npi.

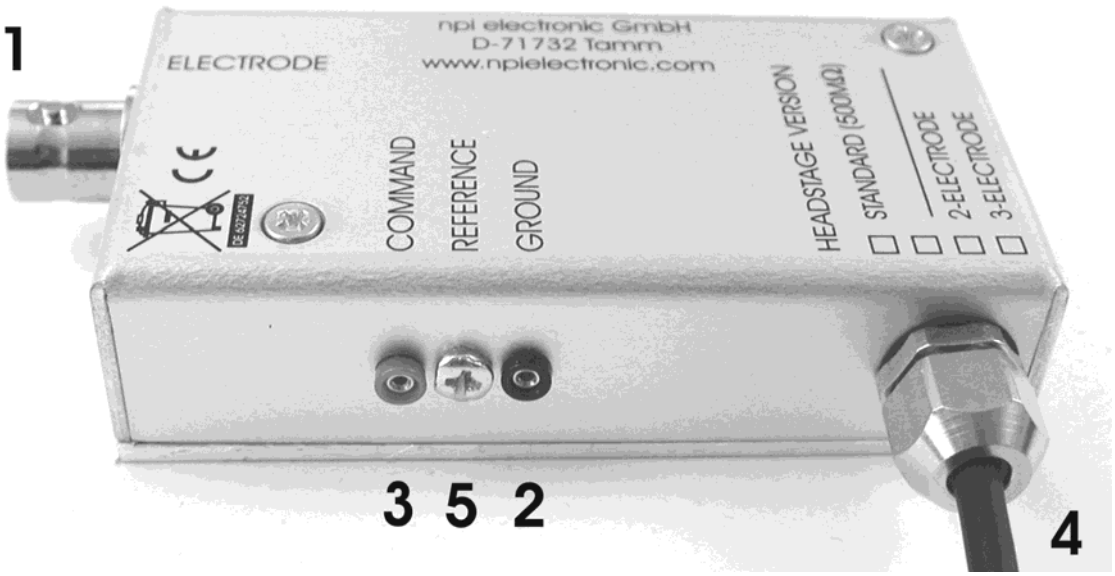

Figure 5: Headstage of the VA-10M

6.1. Headstage Elements

1 BNC connector for the electrode holder
2 GROUND: ground
3 COMMAND: command potential output
4 headstage cable to amplifier
5 REFERENCE: not installed

In the 2-electrode headstage the REFERENCE is not installed. The table indicates whether the headstage is equipped with the standard feedback resistor (500 MΩ) or with a different one. It is also marked whether the headstage is in 2-electrode or in 3-electrode configuration.

The electrical connections are made like in a conventional patch-clamp headstage (e.g. the headstage of the EPC-7 (Heka elektronik, Lambrecht, Germany).
The carbon-fiber electrode fits into the BNC connector of the headstage (#**1**, Figure 5). An electrode holder (optional) gives additional mechanical stability. Ask npi for details. GROUND provides the ground and is linked to the bath, e.g. via an Ag-AgCl pellet. COMMAND provides the command potential at the electrode and remains usually open, but

it can be used to optimize the measurements by connecting it to an electrode shield (see Ogden (1994) for setting up a driven shield configuration). The headstage is attached to the amplifier with the headstage cable (see #**4,** Figure 5) and an 8-pole connector. For maximal flexibility the headstage is mounted on a plastic plate by customized screws. Thus, the user can modify the mounting plate according to his needs, e.g. to mount the headstage to a micromanipulator.

Note: The shield of the BNC connector and the enclosure of the headstage are linked to the command potential output and must not be connected to ground.

Caution: Please always adhere to the appropriate safety precautions (see chapter 1). Please turn power off when connecting or disconnecting the headstage from the HEADSTAGE connector!

7. Operation

7.1. Setting up the VA-10M

The VA-10M EPMS amplifier is shipped as a plug-in unit for the EPMS-07 modular system and equipped with a small headstage with a BNC connector. When the system arrives the headstage will not be connected to the cabinet.

For biological voltammetric measurements the experimental setup typically consists of a microscope located within a Faraday cage to minimize noise pickup. A manipulator is used for positioning the voltammeter headstage with an attached electrode, so that the electrode tip is near the cell(s) to be studied.

After installing the VA-10M plug-in unit in the EPMS-07 housing (see also chapter **Fehler! Verweisquelle konnte nicht gefunden werden.**) mount the headstage to the manipulator.

Important: Always turn power off when connecting or disconnecting the headstage.

To facilitate noise reduction of the setup, the faraday cage and the microscope may be connected to the INTERNAL GROUND located on the back of the EPMS cabinet. Needless to say, grounding for low noise is an art. If you are not familiar with the principles of low noise connections, you should consult the local electrophysiology expert or electrical engineer (see also chapter **Fehler! Verweisquelle konnte nicht gefunden werden.**).

- ❑ Before turning on power, set the switches at the front panel to the following positions:

  Gain (#**3**, Figure 4): 1 mV/pA
  LP Filter (#**5**, Figure 4): 5k
  +/0/- (#**8**, Figure 4): 0

- ❑ Turn power on. The reading of the displays in the modules are an indicator for a working power supply.

As mentioned above the VA-10M can be used for DC amperometry, taking advantage of the internal voltage source, or it can be used with user-supplied external waveforms, e.g. for cyclic voltammetry.

- ❏ The LCD display should read 0. Use the COMMAND potentiometer (#**7**, Figure 4) to apply a constant voltage for DC amperometry. The 3-position polarity switch can be set to "+' or "-", depending on the polarity of the desired command potential. The command voltage is displayed at the LCD in mV. This voltage is applied to the electrode mounted on the headstage.
- ❏ If you intend to read the signal from the VA-10M into a data acquisition system, connect a BNC cable from the acquisition system to FILT. OUTPUT (#**2**, Figure 4) or UNFILTERED OUTPUT (#**1**, Figure 4). Additionally, you can monitor the GAIN setting by connecting a BNC cable from the acquisition system to GAIN MONITOR (#**11**, Figure 4).
- ❏ If you intend to use an external voltage source you need to connect your external voltage source to (**9**) INPUT /10 connector. Remember that the input voltage will be scaled down by a factor of 10 at the headstage. Note that, when an external voltage source is used, the 3-position toggle switch controlling the internal voltage source should be set to "0", unless you want to sum the external voltage with the internal voltage source.

Important: The voltage at the electrode is always the sum of the voltage at INPUT /10 connector and the setting at the COMMAND voltage potentiometer.

The VA-10M is now ready for measurements.

7.2. Testing Basic Functions of the VA-10M

All tests should be made in a noise free environment (e.g. Faraday cage or metal box connected to GROUND). Please be careful, the headstage is sensitive to electrostatic discharges (see also chapter 1). Please note that the headstage enclosure is NOT connected to GROUND, it is connected to the COMMAND signal applied to the microelectrode.

Special notice for 3-electrode headstage: The 3-electrode headstage differs from the standard headstage in having an additional 1 mm electrode connector (REFERENCE) between the GROUND and COMMAND connectors for measuring the bath potential. This signal is processed electronically, so that the command potential is floating with respect to the bath potential. The REFERENCE input must not be open. It has to be connected to GND for these tests.

Before starting the tests, check that if everything is set to zero that there is no offset at the output BNC connectors or digital meter. Also please check that the headstage enclosure (driven shield) is also at zero, e.g. with a digital meter. Then do the following tests:

Open Circuit Test

- ❏ Do not connect anything to the electrode BNC. With no command signals, the current should be zero.
- ❏ Connect a pulse to the command input BNC connector. You should observe only capacitive transients and NO current during the pulse.

DC Accuracy

- ❑ Connect a 100 MΩ or another high value resistor from the electrode BNC to ground.

Caution: Do not use the BNC shield or the headstage enclosure for grounding since they are connected to COMMAND!

- ❑ Apply a command signal of 100 mV DC to the headstage from the COMMAND setting of the voltammeter. Alternatively, connect a DC signal of 1 V to the INPUT /10 BNC connector.

Important: If an external voltage source is used, the 3-position toggle switch controlling the internal voltage source (#**8**) should be set to "0". If the switch is set to "-" or "+", the voltage at the electrode is the sum of the external voltage and the internal voltage source.

- ❑ Check with a digital meter that the headstage enclosure and the shield of the headstage BNC connector are at the COMMAND potential of 100 mV.
- ❑ The COMMAND MONITOR output BNC should provide the correct signal of 1 V.
- ❑ At the current output BNC should be a signal corresponding to Ohm´s Law and multiplied by the selected gain factor.
- ❑ Changing the polarity or magnitude of the command signal must lead to corresponding output signals, especially at the CURRENT OUT BNC connectors (according Ohm´s Law).

Dynamic Test / Frequency Response

For this test a good signal generator with a ramp (triangle / sawtooth) output and an oscilloscope is required.

- ❑ Connect a 1 pF capacitor to the electrode BNC at the headstage. To this capacitor connect a triangle wave generator, with approx. 0.5 V pp and 20-100 Hz.
- ❑ This ramp is transferred into a small current following the formula:

  Ic=C*dU/dt.
  where dU/dt is the slope of the triangle signal (V/sec).
- ❑ Observe the current at the UNFILTERED output using an oscilloscope.

  Note: The observed current is always double since you change from a positive (+) slope to a negative (-) slope [x- (-x) = 2x)].
  Note The amplitude of the current is also influenced by the accuracy of the capacitor and the connecting wires.
- ❑ Start with AMP. and TIME turned into the left most position (counter-clock wise) and increase first AMP. and then TIME by turning the trim pots clockwise. By changing the amplitude and/or frequency you change the dU/dt, and so you can evaluate the range of linearity of the amplifier and also the frequency response.
- ❑ The BOOSTER is set correctly, if the current output is as square as possible. This also depends on the quality of the triangle wave at the 1 pF capacitor.

- The effect of the gain stage and filters can be tested easily, if these tests work.

  Gain stage: When testing the DC accuracy change the setting of the GAIN and observe the correct signal magnitude at the output BNC.
  Filter: If the booster is set correctly connect the oscilloscope to the FILTERED output and change the filter corner frequency. You should see the changes on the shape of the pulses.

7.3. Carbon-Fiber Electrodes

Most voltammetric measurements in today's biological investigations involve the use of carbon-fiber electrodes. These electrodes can be purchased or you can make your own. For use with the VA-10M voltammeter the electrodes must fit to the BNC connector at the input of the amplifier. Two types of connection are commonly used:

1) direct connection via a BNC pin that is soldered onto the end of the electrode or
2) connection via a metal/liquid junction, for example using a 3 M KCl solution to interface the end of a carbon fiber to a Ag/AgCl wire.

For the first type of connection no special holder is required. For the metal/liquid junction type a special electrode holder must be used. For some electrodes a patch-pipette holder is adequate.
Carbon fiber disk microelectrodes with small diameter (5-10 μm range) can be obtained from npi or ALA Scientific Instruments. The electrodes are manufactured using an anodic electrophoretic insulation method (Schulte, A. and R. Chow, 1996, Anal. Chem. 68, 3054-3058).

7.4. Reference- / Counterelectrode

The counter electrode used for biological measurements is typically a Ag/AgCl pellet (a sodium-saturated calomel electrode is sometimes used). The pellet should be immersed into the recording chamber and connected via a thin wire to the ground input of the headstage (#**2**, Figure 5).

7.5. Amperometric Measurements

For high time resolution measurements of transmitter release from single vesicles DC amperometry is the appropriate approach. In this approach, the carbon-fiber electrode is energized with a command potential that exceeds the redox potential of the transmitter being studied. In practice, a command potential of equal to or greater than +650 mV is sufficient for measurements of all major oxidizable transmitters that have been studied to date.
When generating a command potential for DC amperometry, there should be no control voltage at the INPUT /10 BNC. The 3-position command toggle switch (#**8**, Figure 4) should be set, for example to the "+" position. Then, the desired potential can be dialed in with the 10-turn potentiometer. As indicated above +650 mV is sufficient for most measurements with cells.
The amperometric signal is diffusion based. Thus, the distance between the carbon-fiber electrode detecting face and the cell surface must be kept to a minimum. For maximum signal size and most rapid kinetics it is possible to touch the cell membrane with the electrode.

7.6. Cyclic Voltammetry

In order to facilitate the identification of the transmitter being released, it is possible to use various voltage waveforms. One common approach is to apply fast voltage ramp potentials, i.e. to perform fast cyclic voltammetry.
For this application, it is necessary to use an external voltage source connected to the INPUT /10 connector (#**9**, Figure 4) at the front panel of the VA-10M. Because one has to relate the measured current to the applied instantaneous voltage, the current and the applied voltage should be recorded simultaneously with a data acquisition system.

8. Literature

VA-10 typical recordings

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9. Technical Data

Headstage:

Input voltage range:	±1200 mV
Operating voltage:	±12 V
Enclosure:	Size: 40 x 70 x 20 mm, driven shield (COMMAND potential) mounting plate: 50 x 70 mm, not conducting
Electrode connector:	BNC with driven shield (COMMAND potential)
Ground connector:	1 mm connector (black)
Driven shield output:	1 mm connector (red)
Input resistance:	$>10^{13}$ Ω
Current range:	±20 nA max.
Feedback resistor:	500 MΩ
Noise:	<1 pA
LP FILTER:	8-pole Bessel FILTER, attenuation: -48 dB/octave, corner frequencies: 100, 300, 500, 1k, 3k and 5k Hz
GAIN:	6-position rotary switch (x0.5, x1, x2.5, x5, x10, x25 mV / pA)
Voltage source:	COMMAND VOLTAGE set by 10-turn potentiometer, range: ±1000 mV, polarity selectable with polarity switch (+, 0, -), display XXXX mV

OUTPUTS:

Resistance:	250 Ω
FILTERED:	BNC connector, sensitivity selectable with GAIN switch, FILTERED with LP Bessel FILTER
UNFILTERED:	BNC connector, sensitivity selectable with GAIN switch

INPUTS:

Resistance:	1 MΩ
COMMAND VOLTAGE:	INPUT BNC connector, sensitivity: /10

MONITORING:

Impedance:	250 Ω
GAIN:	BNC connector, 1 V per step, +1 V…+6 V
COMMAND:	BNC connector, sensitivity: x10

EPMS-07 SYSTEM

Power requirements:	115/230 V AC, 60/50 Hz, fuse 2 A / 1 A, slow, 45-60 W (dependent on the modules plugged in)
Dimensions:	19” rackmount cabinet, 3U high (1U = 1 ¾” = 44.45 mm)

10. VA-10M with 3-Electrode Headstage

The 3-electrode headstage differs from the standard headstage in having an additional 1 mm electrode connector (REFERENCE) between the GROUND and COMMAND connectors for measuring the bath potential. This signal is processed electronically, so that the command potential is floating with respect to the bath potential. Therefore, the command potential is independent from any bath potential that may occur. Usually an Ag-AgCl silver electrode or pellet is used for measuring the bath potential.

Important: If REF is not used, REF must be connected to GROUND.

Figure 6: **VA-10** 3 electrode headstage with CFE electrode holder (optional)

Reference for typical application:

Marinesco, S. and Carew, T. J. (2002). Serotonin Release Evoked by Tail Nerve Stimulation in the CNS of *Aplysia*: Characterization and Relationship to Heterosynaptic Plasticity. J.Neurosci. **22**(6), 2299–2312.